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Validity of $\dot{V}O_{2\,max}$ in predicting blood volume: implications for the effect of fitness on aging

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Convertino, Victor A., and David A. Ludwig. Validity of $\dot{V}O_{2\,max}$ in predicting blood volume: implications for the effect of fitness on aging. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R1068–R1075, 2000.—A multiple regression model was constructed to investigate the premise that blood volume (BV) could be predicted using several anthropometric variables, age, and maximal oxygen uptake ($\dot{V}O_{2\,max}$). To test this hypothesis, age, calculated body surface area (height/weight composite), percent body fat (hydrostatic weight), and $\dot{V}O_{2\,max}$ were regressed on to BV using data obtained from 66 normal healthy men. Results from the evaluation of the full model indicated that the most parsimonious result was obtained when age and $\dot{V}O_{2\,max}$ were regressed on BV expressed per kilogram body weight. The full model accounted for 52% of the total variance in BV per kilogram body weight. Both age and $\dot{V}O_{2\,max}$ were related to BV in the positive direction. Percent body fat contributed <1% to the explained variance in BV when expressed in absolute BV (ml) or as BV per kilogram body weight. When the model was cross validated on 41 new subjects and BV per kilogram body weight was reexpressed as raw BV, the results indicated that the statistical model would be stable under cross validation (e.g., predictive applications) with an accuracy of ± 1,200 ml at 95% confidence. Our results support the hypothesis that BV is an increasing function of aerobic fitness and to a lesser extent the age of the subject. The results may have implication as to a mechanism by which aerobic fitness and activity may be protective against reduced BV associated with aging.

maximal oxygen uptake; age; body surface area; body fat

SEVERAL INVESTIGATORS HAVE reported relatively high correlations between maximal oxygen uptake ($\dot{V}O_{2\,max}$) and blood volume (BV) (9, 11, 21, 24, 28, 29, 33), suggesting that BV may be predicted from, and contribute significantly to, $\dot{V}O_{2\,max}$. This relationship was not unexpected since an expansion of BV typically accompanies an increase in $\dot{V}O_{2\,max}$ with exercise training (9). However, other investigations produced lower correlations between $\dot{V}O_{2\,max}$ and BV (28) or increased $\dot{V}O_{2\,max}$ without alteration in BV (28, 30, 32, 38). These studies provide evidence that aerobic capacity may not necessarily be related to circulating vascular volume. Unfortunately, interpretation of results from these experiments may have been heavily influenced by the small sample size (11, 28), methodological differences between control and experimental groups (32), posture (27), and gravity (38) conditions during the measurement of BV and $\dot{V}O_{2\,max}$. When sample size was adequate, regression techniques did not include multiple variables (21, 28, 33), or significant variability may have been introduced by inclusion of BV and $\dot{V}O_{2\,max}$ data into models that were measured using a mixture of different techniques (28). Many of these studies used data that were collected for other purposes (i.e., secondary data sets) or that were collected on a restricted range of subjects (e.g., college-age subjects). Additionally, evaluation of these regression models was accomplished by using the same data that were originally used to construct these models and without cross-validation with different data sets. Taken together, these limitations suggest that further studies designed to systematically define the relationship between $\dot{V}O_{2\,max}$ and BV are warranted.

It was therefore the purpose of this investigation to examine the relationship between BV and $\dot{V}O_{2\,max}$ while controlling for covariates known to be related to both variables. This was accomplished by constructing a multivariate statistical model using data that were specifically collected for this purpose and cross validating the resulting model using new observations.

METHODS

Subjects

Sixty-six healthy, nonsmoking, normotensive Caucasian men with a mean ± SD age of 37 ± 10 yr, a mean height of 178 ± 6 cm, a mean weight of 76.7 ± 8.5 kg, and a mean $\dot{V}O_{2\,max}$ of 47.5 ± 10.3 $ml \cdot kg^{-1} \cdot min^{-1}$ gave written consent to participate in this study. All subjects were thoroughly briefed on the test procedures before consenting to participate. The complete study protocol was approved by the Institutional Human Research Review Boards at the National Aeronautics and Space Administration (NASA)-Ames Research Center (Mountain View, CA) and NASA-Kennedy Space Center. Subjects were volunteers who were recruited

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from a general population that was large and diverse, ranging from unskilled laborers to doctoral-level research scientists. Participation in the study was based on a screening evaluation that consisted of a detailed medical history, physical examination, complete blood count, a panel of blood chemistry analyses, urinalysis, a resting and treadmill electrocardiogram, pulmonary function tests, and body composition. Subjects were disqualified as participants if any of the above medical markers indicated values outside normal ranges.

Sampling Plan and Cross-Validation

To guard against the problem of restricted variation across both independent and dependent variables, the sampling plan was set up with age stratification to assure that a wide variety of ages were included. Subjects were recruited from each of these age strata as possible candidates for this study. This sampling plan produced an age range from 18 to 55 yr.

A statistical power analysis was performed to determine the sample size needed to detect an approximate change in full model R^2 of 0.10 for any effect in the model given a type I error rate of 5%. It was determined that a sample of at least 60 subjects would be needed for 90% power for any effect when the full model R^2, estimated from previous research, was ~0.50 (7). The final sample consisted of 66 subjects.

Data on 41 additional subjects available from previous research studies were used to cross validate the model derived from the original group of 66 subjects. Subjects in the cross-validation group met all of the informed consent and medical entrance criteria described above. All of the cross-validation subjects were nonsmoking normotensive men, with a mean ± SD age of 36 ± 4 yr, a mean height of 177 ± 5 cm, a mean weight of 80.2 ± 11.7 kg, and a mean $\dot{V}O_{2\,max}$ of 44.5 ± 7.0 $ml \cdot kg^{-1} \cdot min^{-1}$.

Independent Effects and Measurements

$\dot{V}O_{2\,max}$. A treadmill protocol consisting of stepwise elevations in grade and speed until each subject reached volitional exhaustion (4) was used to elicit $\dot{V}O_{2\,max}$. Subjects breathed through a low-resistance valve, and the volume and composition of the expired gas was collected continuously and analyzed for the fractions of mixed expired oxygen ($\dot{V}O_2$) and carbon dioxide. The $\dot{V}O_2$ calculated from these data collected during the final 30 s of the treadmill protocol was used to identify $\dot{V}O_{2\,max}$.

Body surface area. Height was measured to the nearest centimeter using a standard medical scale height ruler, and body weight was measured to the nearest ±5 g with a digital-load cell scale (Sartorius Scale, Goettingen, Germany). Body surface area (BSA) was calculated from height and weight using the following standard surface area formula of Du Bois and Du Bois (12)

$$BSA\ (m^2) = 0.007184 \times height\ (cm^{0.725}) \times weight\ (kg^{0.425})$$

Percent body fat. Body density and percent body fat (PBF) were determined by underwater densitometry, with correction for residual lung volume (36). After the measurement of nude body weight in air, each subject was seated in an aluminum weighing seat that was suspended in a tank of water (temperature = 34°C). The subject was instructed to exhale completely and expel all of the air from his lungs while he lowered his head toward his legs in a tucked position until he was completely immersed. Underwater weighings were repeated until maximal underwater weight was maintained. Residual lung volume was measured by the nitrogen washout method with an Ohio model 700 nitrogen analyzer. Lean body mass was derived by subtracting calculated total body fat from total body weight.

Age. Age was measured in years from the last birthday and was obtained by interviewing the subject.

Dependent Effect and Measurements

BV. Total BV was calculated from the plasma volume and peripheral venous hematocrit measurements. BV was standardized for body size by dividing total BV by body weight in kilograms (BV per kilogram body weight). Plasma volume was determined by a modified dilution technique (10, 17) using sterile solutions of Evans blue dye contained in 10-ml ampules (The New World Trading, DeBary, FL). After each subject was stabilized in the supine position for 30 min, a preinjection control blood sample was drawn followed by an intravenous injection of 11.5 mg of dye diluted with isotonic saline solution (2.5 ml) that was administered through a sterile 0.45-μm filter. One milliliter of plasma from a 10-min postinjection blood sample was passed through a wood-cellulose powder (Solka-Floc SW-40A) chromatographic column so that the dye could be absorbed. The absorbed dye was eluted from the column using a 1:1 water-acetone solution (pH = 7.0) and was collected in a 10-ml volumetric flask. The postinjection solution was compared with 1-ml samples from a preinjection time (zero control) and a standard dye solution (1:50 dilution with distilled water), and all samples were read at 615 nm with a spectrophotometer. With the use of these procedures in our laboratory, the test-retest correlation coefficient for BV was 0.969 (n = 12) and the average changes were 82 ml (average %change = 1.5%, n = 17), 75 ml (average %change = 1.5%, n = 19), 56 ml (average %change = 1.1%, n = 23), and 25 ml (average %change = 0.7%, n = 7) when measurements were determined 4, 8, 15, and 330 days apart, respectively (10, 17).

Statistical Methods

A multiple linear regression model was used to determine whether BV per kilogram body weight could be predicted from age, PBF, and $\dot{V}O_{2\,max}$. A full model regression using all three independent variables was constructed, and the unique contribution of each variable to the prediction of BV per kilogram body wt was assessed using regression leverage plots, partial correlations, and R^2 values. These plots and associated partial correlation coefficients were used to reflect the relationship between each of the predictor variables and BV per kilogram body weight after the variance shared between BV per kilogram body weight and the other predictors in the model had been removed. Multicollinearity was then checked for each independent variable in the model by calculating the variance inflation factor (VIF; see Ref. 26). The tenability of the underlying model assumptions was then assessed followed by tests on the individual parameters using partial F statistics. All statistical probabilities associated with statistical tests are given as exact probabilities and reflect the chances of observing a parameter(s)-shared variance with the dependent variable given an estimated error-only system.

The stability of the final multiple regression model and subsequent estimates was tested using data from 41 subjects not included in the initial model formulation (i.e., cross validation). The process of cross validation assesses the stability and utility of the model when applied to data other than that which derived it. An additional check of model stability was performed by fitting a second multiple regression model using absolute BV (ml) as the dependent variable while adding BSA as an independent effect (i.e., model repa-

Table 1. *Subject descriptive statistics*

Variable		Minimum	Maximum
Age, yr	37 ± 10	18	55
Body surface area, m^2	1.94 ± 0.13	1.70	2.23
Percent body fat	18.6 ± 6.6	7.8	33.7
$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	47.5 ± 10.3	27.6	71.8
Blood volume, ml	5,738 ± 823	3,997	7,780
Blood volume, ml/kg	75.4 ± 12.1	50.5	126.4

Data are means ± SD; n = 66 subjects; $\dot{V}O_{2max}$, maximal O_2 uptake.

rameterization). All statistical procedures were performed using JMP Statistical Discovery Software (Version 3.2, SAS Institute, Cary, NC).

RESULTS

Full Model Estimation

Descriptive statistics for all of the variables used in the modeling of BV are presented in Table 1. Minimum and maximum values listed in Table 1 reflected a wide range and good distributional properties (e.g., symmetric box plots, etc.) and assured adequate variation in all variables. The univariate correlation matrix for all six variables used in the modeling is presented in Table 2 for descriptive purposes.

The summary of fit for the full model using BV per kilogram body weight as the dependent variable is presented in Table 3. The full model accounted for 54% of the total variance in BV per kilogram body weight [$F(3,62) = 23.9$, $P = 0.0001$]. The partial r statistic listed in Table 3 reflects the correlation of each of the independent variables after BV per kilogram body weight had been corrected (adjusted) for the other effects in the model. The percent reduction in error (PRE) for each variable in the full model listed in Table 3 equals the square of the partial r statistic multiplied by 100 and reflects the PRE that would result if that particular variable were added to a model consisting only of the other two remaining effects. The change in full model R^2 (ΔR^2) resulting from the omission of a particular variable from the three-variable model is also listed in Table 3. For this full model, omission of age and $\dot{V}O_{2\,max}$ would reduce the full model R^2 by 0.09 (PRE = 16%) and 0.18 (PRE = 27%), respectively. This result is confirmed by the VIF statistics presented in Table 3, since VIF values departing from 1.0 indicate some degree of multicollinearity for a particular effect in the model. PBF and $\dot{V}O_{2\,max}$ demonstrated slightly elevated VIF values due to their high intercorrelation (−0.81, see Table 2).

Table 2. *Univariate correlation matrix*

	Age	BSA	PBF	$\dot{V}O_{2max}$	BV, ml	BV, ml/kg
Age	1.00	−0.04	0.45	−0.46	−0.03	−0.05
BSA		1.00	0.23	−0.34	0.39	−0.32
PBF			1.00	−0.81	−0.31	−0.56
$\dot{V}O_{2max}$				1.00	0.37	0.67
BV, ml					1.00	0.72
BV, ml/kg						1.00

Data are for n = 66 subjects. BSA, body surface area; PBF, percent body fat; BV, BV.

Table 3. *Full model summary of fit for BV*

Effect	Estimate	Partial F Ratio	Partial r	ΔR^2	PRE	VIF
Intercept	23.51 ± 13.75					
Age	0.43 ± 0.12	11.86	0.40	0.09	16	1.30
PBF	−0.23 ± 0.27	0.70	−0.11	0.01	1	2.96
$\dot{V}O_{2max}$	0.85 ± 0.18	23.32	0.52	0.18	27	3.00

Data for estimate are means ± SE; n = 66 subjects. Units are ml/kg. PRE, percent reduction in error; VIF, variance inflation factor. Full model R^2 = 0.54, mean squared error = 71.39. $P(1,62) \leq 0.001$ for age and $\dot{V}O_{2max}$.

The partial F ratios for each effect in the model are also presented in Table 3. The F ratios reflected the degree of ΔR^2 uniquely attributable to a particular variable in the model, with a ratio of 1.0 indicating that additional variance accounted for by an effect was no greater than that variance attributed to experimental error (i.e., the estimated "noise" in the system). All independent variables, with the exception of PBF, had substantial partial F ratios [$F(1,62) \geq 11.86$, $P = 0.001$].

Model Reduction

Given the results of the multivariate regression analysis, PBF was dropped from the model. The remaining parameters of this reduced model were then reestimated and evaluated for multicollinearity. The results are presented in Table 4. The reduced model demonstrated advantages over the full model. Because PBF was removed, the VIF statistics approached one, and the unique effects of each of the independent variables are more defined with no loss in overall explained variance [$R^2 = 0.53$, $F(2,63) = 35.6$, $P = 0.0001$]. The results indicate that $\dot{V}O_{2\,max}$ adds the most weight in the model for prediction of BV per kilogram body weight, contributing a percentage reduction in error of ~53%. After $\dot{V}O_{2\,max}$ is considered, age accounts for an additional 9% of the total variation in BV. The prediction model for BV per kilogram body weight using age and $\dot{V}O_{2\,max}$ is as follows

$$\text{predicted BV (ml/kg)} = 14.51 + 0.411\,(\text{age}) + 0.962\,(\dot{V}O_{2\,max})$$

Table 4. *Reduced model summary of fit for BV*

Effect	Estimate	Partial F Ratio	Partial r	ΔR^2	PRE	VIF
Intercept	14.51 ± 8.54					
Age	0.41 ± 0.12	11.30	0.04	0.09	15	1.27
$\dot{V}O_{2max}$	0.96 ± 0.11	70.90	0.73	0.53	53	1.27

Data for estimate are means ± SE; n = 66 subjects. Units are ml/kg. Full model R^2 = 0.53, mean squared error = 71.05. $P(1, 63) \leq 0.0013$ for all effects.

Figure 1, *A* and *B*, shows the relationship of each of the independent variables with BV per kilogram body weight after adjusting for the other variables in the model. These scatter plots (partial leverage plots) reflect the multivariate association of each independent variable with BV per kilogram body weight after the effects of the other variables in the model have been considered. The degree of relationship observed in each of these plots was quantified by the partial correlation. A 95% bivariate normal ellipse was added to each plot to help further define the multivariate relationship of each independent variable with BV per kilogram body weight. Figure 2*A* gives the full model prediction against the observed BV per kilogram body weight and reflects the overall accuracy of prediction for the two-variable model. Figure 2*A* also supports the tenability of the statistical assumptions regarding the normality and homoscedasticity of the prediction errors.

Because BV is expressed per kilogram of body weight, the prediction accuracy [root mean square error (RMSE)] is presented in compound units (i.e., a compound function of both BV and body weight). However, if predicted BV per kilogram body weight is unstandardized through postmultiplication by weight, the estimated prediction error is ~598 ml (1,200 ml at 95% confidence).

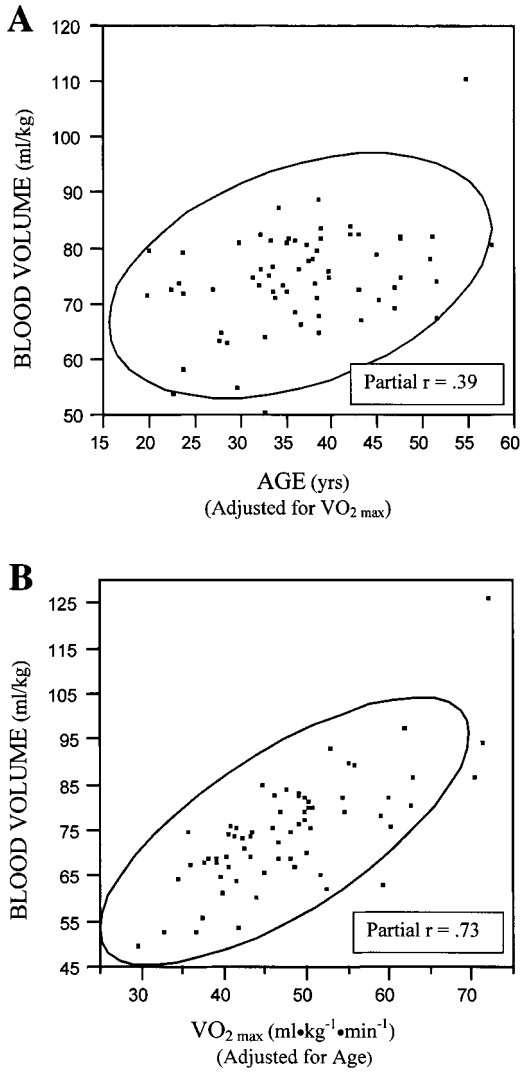

Fig. 1. Partial leverage plots for age (*A*) and maximal oxygen uptake ($\dot{V}O_{2max}$; *B*) as predictors of BV per kg of body wt.

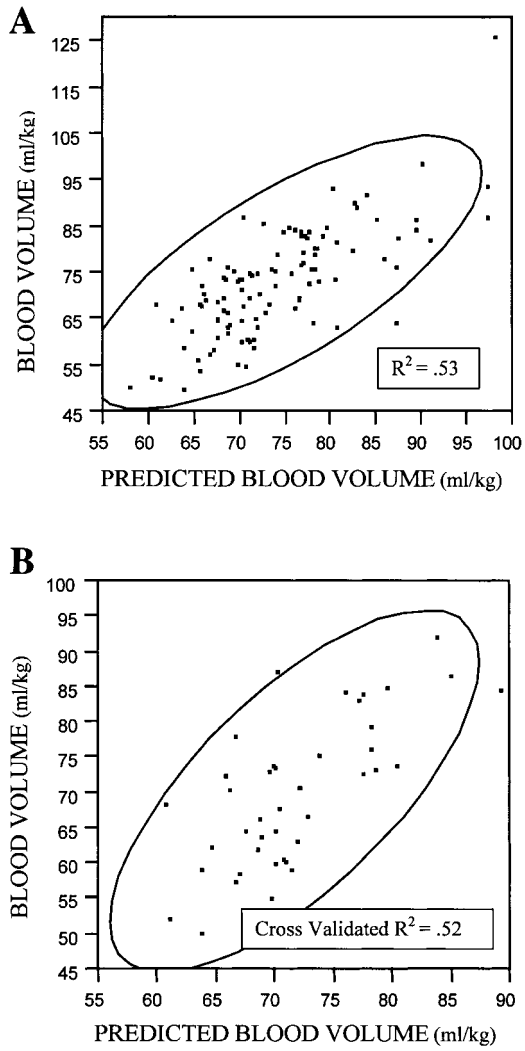

Fig. 2. Relationship between actual and predicted BV per kg of body wt from age and $\dot{V}O_{2max}$ in the original sample (*A*) and in 41 new individuals (*B*).

Cross Validation

To test the stability of the statistical estimates and the precision of the prediction equation, the model was applied to other data used to derive the model. The prediction model was applied to data from 41 new subjects, and various aspects of fit and prediction accuracy were evaluated. The full model R^2 of 0.52 under cross validation was similar to the 0.54 from the original estimation. The cross-validated RMSE for the full model was 7.5 ml/kg compared with 8.4 ml/kg from the original estimation. When the prediction errors were converted to milliliters by postmultiplication of body weight, the cross-validated RMSE was ~590 ml. Thus the unbiased estimated individual error of prediction

remained at approximately ±1,200 ml (~95% confidence). Only one (2.4%) of the 41 subjects used in the cross-validation had a predicted BV that was >1,200 ml from the observed BV.

Figure 2*B* shows the predicted BV per kilogram body weight vs. the actual BV per kilogram body weight when the statistical model derived from the initial 66 subjects was applied to the 41 new individuals. The overall appearance of this scatter plot is similar to that in Fig. 1*A*, reflecting stability of model performance when data from new individuals are applied.

Model Reparameterization

As an additional check of model stability, we fit a second model using absolute BV (ml) as the dependent variable and included BSA as an independent effect. Originally, BSA was not included in the modeling of BV per kilogram body weight since both BV per kilogram body weight and BSA were derived from body weight. When the model is fit and reduced for prediction of absolute BV, age, BSA, and $\dot{V}O_{2\,max}$ explained 52% of the variance in BV. As in the original model, PBF explained <1% of the variance in BV. BSA and $\dot{V}O_{2\,max}$ carried approximately equal weight in the model, with both resulting in full model R^2 reduction of ~0.37 when dropped from the full model. Age accounted for a reduction of 0.08 in full model R^2. The prediction error was virtually identical between the BV per kilogram body weight and absolute BV models. For the absolute BV model, the RMSE was 594 ml, resulting in an approximate prediction interval of ±1,200 ml. Therefore, for any given individual, the error in a prediction of absolute BV from age, BSA, and $\dot{V}O_{2\,max}$ was approximately ±1,200 ml (95% confidence). The final model for predicting absolute BV is as follows

$$\text{predicted BV (ml)} = -6578.8 + 28.7\ (\text{age}) + 4326.3\ (\text{BSA}) + 59.7\ (\dot{V}O_{2\,max})$$

When both models are applied to the cross-validation data (41 new subjects) and predicted BV per kilogram body weight is converted to milliliters, the correlation between the two sets of predicted values was 0.98.

DISCUSSION

The primary finding of the present investigation was that $\dot{V}O_{2\,max}$ represented a significant predictor of BV. The univariate correlation coefficient of 0.67 between $\dot{V}O_{2\,max}$ and BV calculated from the data of 66 male subjects in the present study was consistent with moderately high zero-order correlation coefficients between $\dot{V}O_{2\,max}$ and BV (ml/kg body wt) reported from several experiments (Table 5). These relationships indicate a direct influence of BV on aerobic capacity and/or vice versa. However, a major limitation to the application of univariate correlations is that they do not reflect the manner in which numerous variables relate to each other and/or to BV after the variances associated with other variables have been accounted for (i.e., multivariate relationships). Although previous investigations have assessed relationships between $\dot{V}O_{2\,max}$ and BV with the use of zero-order correlation coefficients (9, 11, 21, 24, 28, 29, 33) or cross-sectional comparisons of mean values (1, 3, 9, 16, 22, 35), we are unaware of any studies that have applied a statistical approach that considered the collinearity between these and other variables. Therefore, a unique feature of the present investigation was the development of a predication model using multivariate regression statistics in an attempt to account for various covariance parameters associated with $\dot{V}O_{2\,max}$ and BV. Our results confirm that $\dot{V}O_{2\,max}$ is a primary contributor to the prediction of BV.

Table 5. *Comparison of univariate correlation coefficients between total BV and $\dot{V}O_{2\,max}$ reported in the literature*

Investigation	Sample Size	*r*
Present study	66	0.67
Convertino (9)	97	0.78
Davy and Seals (11)	14	0.73
Jones et al. (21)	52	0.65
Mack et al. (24)	11	0.92
Sawka et al. (28)	51	0.52
Schmidt et al. (29)	311	0.91
Stevenson et al. (33)	30	0.79

$P < 0.05$ for all *r* values.

Contrary to the findings that lean body mass provides a strong index to predict BV (2, 28), body density did not account for any additional variation in BV in the present investigation once BV was expressed per unit of body weight or BSA was considered. It has been hypothesized that, because adipose tissue is less vascular than fat-free mass, body density should account for additional variation in BV beyond that which can be explained by overall size of the individual (19). Although theoretically reasonable, the current findings do not support this notion and suggest that body fat does not contribute to the prediction of BV per kilogram body weight after age and $\dot{V}O_{2\,max}$ have been considered. Our data suggest that individuals with high PBF were generally larger and, subsequently, had larger somatotype and more surface area. The observation that PBF was highly related to $\dot{V}O_{2\,max}$ in our subjects (Table 2) was consistent with previous reports (11, 33) and provided another explanation for the failure of PBF to contribute to the model for prediction of BV per kilogram body weight in the present investigation. This conclusion is contrary to that suggested by univariate correlation between BV per kilogram body weight and PBF that indicated a moderate negative relationship (Table 2). Because PBF was positively related to age and negatively related to $\dot{V}O_{2\,max}$ in the present study, there was no additional variance left in the BV per kilogram body weight model to be accounted for by PBF once effects for $\dot{V}O_{2\,max}$ and age had been accounted for. This result indicates that PBF is merely an alias of $\dot{V}O_{2\,max}$ and that PBF has no direct effect on BV.

Before conducting a multiple-regression statistic using PBF as a measure of body density, several other measures of body density and/or body type (including height, body weight, and BSA) proposed by other investigators to be good predictors of BV were investigated for use in our statistical model (2, 14, 15, 18–20, 28, 35). These factors included lean body mass, desirable weight, body mass index, body density, and several indexes involving body weight and height (e.g., Quetelet Index, ponderal index, BSA). Because many of these measures are simple linear transformations of PBF, and to avoid issues of multicollinearity, only one measure of "body density" could be applied to the statistical model. None of these measures explained any more of the variation in BV than PBF or body weight once $\dot{V}O_{2\,max}$ was considered. Because BV was expressed per kilogram of body weight, weight could not be used as an independent variable in the model. Height accounted for <1% of the variance in the prediction of BV per kilogram body weight once age and $\dot{V}O_{2\,max}$ were considered in the model.

Previous investigations have revealed that BV either decreased (6, 11, 15, 21, 31) or was unaffected (8, 34, 36) by age. These findings may represent more of a statistical artifact than a true relationship since statistical analyses that supported the notion that BV decreased with age have been limited to use of simple cross-sectional comparison of mean values between BV and age. When applying a single correlation to our data, we also found a negative relationship between age and BV (Table 2). However, when the correlation between BV and age is partialed for $\dot{V}O_{2\,max}$, the relationship is moderately positive partial correlation = 0.39, Table 4. Therefore, contrary to previous findings, our multivariate model revealed that BV increased with age (Table 3 and Fig. 1*A*). The regression model presented in Table 4 represents one of classic suppression (cooperative) since the effect of age is not apparent unless $\dot{V}O_{2\,max}$ is included in the model. Suppression is a common model for homeostatic mechanisms in biology in which factors such as BV, $\dot{V}O_{2\,max}$, and age occur together and have counteractive effects (7). Therefore, some of the variance in BV cannot be explained unless age and $\dot{V}O_{2\,max}$ are considered jointly. Suppression occurs since, in the univariate correlations, age and $\dot{V}O_{2\,max}$ are related, but only $\dot{V}O_{2\,max}$ is related to BV (Table 2). As a result, the effect of age is "suppressed" unless $\dot{V}O_{2\,max}$ is considered.

The suppression effects acting in the model presented in Table 4 are graphically depicted in Fig. 3, where BV (ml/kg) is plotted against age for all 107 observations made in the present study. Points on this scatter plot have been identified by $\dot{V}O_{2\,max}$ level. Low $\dot{V}O_{2\,max}$ values (<40 ml·kg^{-1}·min^{-1}, 25th percentile) are shown as green dots, high $\dot{V}O_{2\,max}$ values are shown as red squares (>52 ml·kg^{-1}·min^{-1}, 75th percentile), and the remaining middle 50% of the data are shown as blue crosses. When an overall regression line is fit to these data, ignoring $\dot{V}O_{2\,max}$ level, the slope is negative (black line). However, when separate regressions are fit to the data within each $\dot{V}O_{2\,max}$ level (red, green, and blue lines), the relationship between age and BV is positive. The overall negative trend is a result of younger subjects having higher $\dot{V}O_{2\,max}$ values (Fig. 3, *top left*), whereas older subjects have lower $\dot{V}O_{2\,max}$ values (Fig. 3, *bottom right*). When all subjects are considered together, the negative (left) side of the regression line is pulled up by the younger subjects, and the positive (right) side of the regression line is pulled down by the older subjects. When $\dot{V}O_{2\,max}$ is not considered, the relationship between age and BV appears to be negative; when $\dot{V}O_{2\,max}$ is considered in the regression, the relationship between BV and age is actually positive.

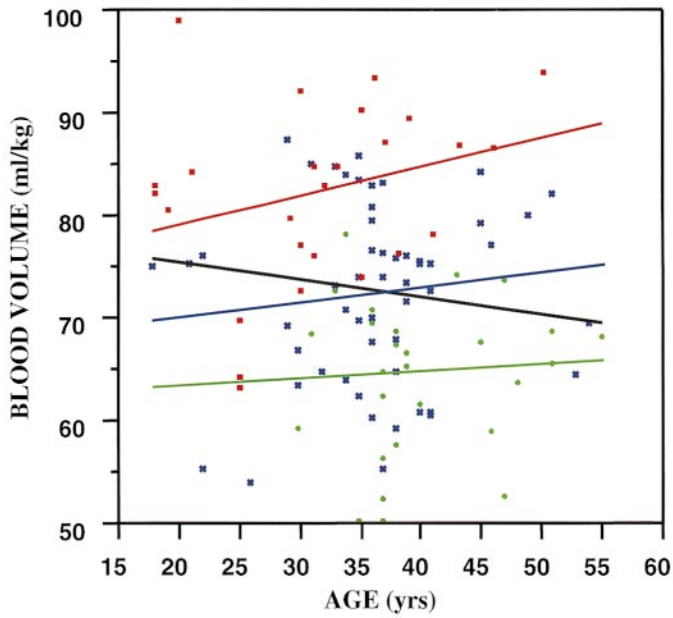

Fig. 3. Scatterplot of age and BV with points identified by high (red squares), medium (blue crosses), and low (green dots) levels of $\dot{V}O_{2\,max}$. Overall regression line (black) and individual regression lines within each $\dot{V}O_{2\,max}$ level (green, blue, red).

Our conclusions are in direct conflict with those of Sawka et al. (28), who concluded that $\dot{V}O_{2\,max}$ does not correlate with BV. We believe that the difference in the findings between the two investigations is primarily attributable to the statistical approach and sample used by Sawka and co-workers. As previously discussed, the BV model is one of cooperative suppression in which the relative contributions of age and $\dot{V}O_{2\,max}$ to the prediction of BV can only be evaluated when age and $\dot{V}O_{2\,max}$ are included in a multivariate model at the same time. Rather than applying a multivariate model analysis, Sawka et al. used a series of simple regression models (i.e., univariate) in an attempt to find a suitable model. Unfortunately, univariate models are notoriously weak when quantifying patterns of association and/or causal relationships between variables (13). Even with proper specification of the model, we believe the sample of subjects used by Sawka and co-workers was biased to highly fit individuals (41.9–65.4 ml·kg^{-1}·min^{-1}) of a relatively young age group (18–35 yr). This restricted range of both age and

$\dot{V}O_{2\,max}$ may have effectively masked the interrelationship that actually exists between $\dot{V}O_{2\,max}$, age, and BV (7).

The present experiment was designed to assure adequate variation in all variables, especially age. Another unique feature of our multivariate model was the use of an independent subject population with a large sample size to test the validity of the model. The full model R^2 of 0.52 as well as the scatter plot (Fig. 2*B*) under cross validation were similar to the R^2 of 0.53 and scatter plot (Fig. 2*A*) from the original estimation. These comparisons indicate that overall the model performed well when it was applied to new individuals. $\dot{V}O_{2\,max}$ proved to be the single best predictor of BV (ml/kg) and when considered negates the effect of PBF (i.e., body density). Although the effect was somewhat small, when $\dot{V}O_{2\,max}$ is held constant, BV tends to increase with age. The prediction accuracy of BV models for standardized (ml/kg) and absolute (ml) values are, for all practical purposes, identical. Although the model parameters differ, the relative information regarding body size remains intact in both models. The absolute BV model is a bit more complex since it requires the additional calculation of BSA, whereas the standardized BV model requires postmultiplication by weight to obtain BV in milliliters. When both models are applied to the cross-validation data (41 new subjects), and predicted BV per kilogram body weight is converted to milliliters, the correlation between the two sets of predicted values was significantly high.

Our experiment was not without limitation. We recognize that the present study represents an observational investigation that was dependent on inclusion of relevant independent variables. Thus it is possible that our model could have presented specific bias in the estimates for regression coefficients of the included independent variables if there were additional variables that had important contribution but were not included in the model. For this reason, we went to extremes to assure that there was adequate variance across both independent and dependent variables and that we considered in our analysis all known variables that have been identified or suggested as important to the prediction of BV (1–3, 5, 6, 8, 9, 11, 14–16, 18–22, 25–28, 31, 32, 34). We then checked our models through cross validation. Given our design characteristics, the observed effects are so large that it is difficult to account for more than a small part of this effect by other variables not considered in this analysis. This does not mean that our conclusions and eventual generalizations are not restricted by our sample (Caucasian males) but that statistical bias in our estimation procedure should be small.

Perspectives

Our findings have particular implications for the effect of physical activity and fitness on aging. Our data support the notion that BV actually increases with age but that this relationship may be masked by a sedentary "Western" lifestyle that can often accompany the aging process. This notion is further supported by the observations that BV can be increased with regular physical activity in elderly individuals to the same relative degree compared with younger people (5) and that reduction in BV associated with aging in sedentary subjects was removed in physically active subjects (21). The contraction of BV may be associated with adverse impacts on risk factors for cardiovascular disease such as elevated low-density lipoprotein cholesterol, increased whole blood viscosity, and stimulation of sympathetic nervous activity (33). In turn, sympathetic hyperactivity is typically reported in patients with essential hypertension and chronic renal failure and is associated with poor prognosis and increased risk of sudden death (23). The relationships between BV, sympathetic activity, and progressive cardiovascular disease may reflect a protective nature of increased BV against development of coronary heart disease (CHD) with aging. If it is true that BV increases with age when a sedentary lifestyle has been removed (21), then perhaps a sedentary lifestyle is actually acting to remove a natural CHD protective factor. For example, if less viscous circulating blood or sympathetic activity results from increasing BV with regular physical activity during the aging process, then various risk factors associated with CHD such as platelet aggregation, arterial thrombosis, or cardiac arrhythmias are less likely to occur. The literature supports the observation that, in the Western world, BV does decrease with age (6, 11, 15, 21, 30). However, our data provide compelling evidence that reduced BV with age may be a result of a sedentary, high-caloric lifestyle rather than the aging process. Therefore, perhaps one of many important benefits of maintaining physical activity and fitness during aging is the resultant expansion of plasma and BV that provides a protective effect against development of cardiovascular disease.

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